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ENVIRONMENTAL RESEARCH 47, 79–94 (1988)

Lead and Osteoporosis: Mobilization of Lead from Bone in Postmenopausal Women

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Received March 1, 1988

Although it has been known that humans accumulate lead in bone, mineralized tissue has been considered primarily as a sequestering compartment and not as a site of toxic action for lead. However, experimental data indicate that bone lead can be released during conditions of demineralization, such as pregnancy and lactation. We have examined lead status in women, before and after menopause, using the NHANES II dataset compiled between 1976 and 1980. In 2981 black and white women there was a highly significant increase in both whole blood and calculated plasma lead concentrations after menopause. The results indicate that bone lead is not an inert storage site for absorbed lead. Moreover, lead may interact with other factors in the course of postmenopausal osteoporosis, to aggravate the course of the disease, since lead is known to inhibit activation of vitamin D, uptake of dietary calcium, and several regulatory aspects of bone cell function. The consequences of this mobilization may also be of importance in assessing the risks of maternal lead exposure to fetal and infant health. © 1988 Academic Press, Inc.

INTRODUCTION

Most of the lead accumulated by humans over time is deposited in bone (Schroeder and Tipton, 1986; Aufderheide *et al.*, 1981), a long term storage site for lead, with a half-life of about 20 years (Rabinowitz *et al.*, 1976). Bone uptake of lead has been assumed to be essentially a sequestering and functionally detoxifying mechanism that effectively removes lead from the bioavailable blood compartment without adverse effect on bone. Although several toxicokinetic studies have challenged this assumption (Pounds, 1984; Manton, 1977), monitoring levels of lead in blood still forms the basis for diagnosis and medical management of persons exposed to lead. This may be of particular concern in medical removal policies in occupational medicine, under which workers' exposure is reduced only for the time required for lead to be reduced in blood, although it is known that bone deposits remain elevated for much longer periods (Christofferson *et al.*, 1986).

The results of the present investigation challenge these assumptions. This study provides evidence that bone lead is in fact subsequently mobilized into blood during conditions of bone demineralization. A major implication of this finding is that even low level lead exposure, over a relatively long time, may result in increased body burdens of lead which would be releasable in toxicologically significant amounts during critical physiological states.

Three major physiological conditions during which bone demineralization can occur are pregnancy, lactation, and osteoporosis. During pregnancy, significant

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bone resorption as been found in women without prior bone disease and without grossly deficient dietary calcium intake (Lamke *et al.*, 1977; Pitkin *et al.*, 1979). In certain pathological cases, clinical osteoporosis may occur during pregnancy and lactation resulting in the loss of as much as 10% of bone mineral content, as measured radiologically (Gruber *et al.*, 1984).

Postmenopausal osteoporosis is a major bone disease which affects millions of older women in the United States. It is associated with increased risks of bone deformation and fractures, which are of particular concern in the elderly population (Riggs and Melton, 1984; Cummings *et al.*, 1985). The extent of mineral depletion from bone in osteoporotic women has been estimated to range from 2 to 10% and occurs usually over the first 2 to 4 years following cessation of menses (Ruegsegger *et al.*, 1984). The mechanisms of postmenopausal osteoporosis are not well understood, but changes in estrogen levels, parathyroid hormone (PTH) release, decreased hormone receptor sensitivity, changes in bone cellular physiology, impaired vitamin D activation, and reduced intestinal absorption of dietary calcium have all been proposed as mechanisms. Some or all of these phenomena may be responsive to the hormonal changes of menopause (Riggs and Melton, 1984; Cummings *et al.*, 1985; Aloia *et al.*, 1985; Tsai *et al.*, 1984).

Because osteoporosis is a condition in which significant bone mineral loss occurs, it was of interest to us to investigate the possibility that lead is mobilized from bone as part of the demineralization process in a well-characterized sample population of postmenopausal women. In addition, mobilization of lead from bone during pregnancy and lactation (Wolff, 1983) is of concern because of recent information on the adverse effects of lead on the fetus and neonate at relatively low levels of exposure, such that even marginal increments in bioavailable lead could significantly increase the risk of adverse outcomes.

Bone has not generally been considered a site of toxic action for lead, and lead exposure has not been proposed as a risk factor for osteoporosis. Nevertheless, lead is a possible candidate for at least a partial role in the etiology of resorptive bone disease. Lead exposure reduces bone formation activity in dogs with blood lead levels between 50 and 80 mcg/100 ml (Anderson and Danylchuk, 1977), and in rabbits with high dose lead exposure (Hass *et al.*, 1967). Lead exposure of young children is associated with decreased growth and stature, which may involve effects on skeletal growth (Huseman *et al.*, 1987; Schwartz *et al.*, 1986; Lauwers *et al.*, 1986). Some of the other well-described toxicological effects of lead are also consistent with the proposed pathophysiology of osteoporosis: lead decreases dietary calcium absorption (Gruden, 1975), and in children interferes with thyrotropin-stimulating hormone release from pituitary (Huseman *et al.*, 1987) and inhibits the 1-hydroxylation of vitamin D (Rosen *et al.*, 1980; Mahaffey *et al.*, 1982; Edelstein *et al.*, 1984).

METHODS

The data collected during the second National Health and Nutrition Examination Survey (NHANES II) were utilized in this study (National Center for Health Statistics, 1981, 1984). NHANES II has been very useful in developing information on the extent of lead exposure in the U.S. population, sources of that expo-

sure, and associations with adverse medical status (for instance, increased systolic and diastolic blood pressure in men (Pirkle *et al.*, 1985), and decreased growth and stature in children (Schwartz *et al.*, 1986)).

With these data, we investigated the following hypotheses related to the mobilization of lead from bone: (1) that postmenopausal women have higher blood levels than premenopausal women (controlling for age) and (2) that pregnancy also mobilizes lead from bone, resulting in less mobilization due to lower bone lead stores in those postmenopausal women who had ever been pregnant. Whole blood levels as measured (see below) were used, as were calculated plasma lead values. Plasma lead is proposed to more accurately reflect the delivered dose to soft tissues. This second analysis was a nonlinear toxicokinetic model of the relationship between blood lead and plasma lead which has been fit to actual plasma lead data (De Silba, 1981) by Marcus (Marcus, 1985). While blood lead is commonly used as a marker for lead exposure, to indicate dose at soft tissue sites, most of the lead in blood is bound to the erythrocyte and relatively less toxicologically available than the plasma component. Overall, plasma lead is nonlinearly related to blood lead because of the finite capacity of the red cell to bind lead at high concentration (De Silba, 1981; Manton and Look, 1984). The calculated dose proportional to plasma lead has been shown to be a better predictor than blood lead of erythrocyte protoporphyrin levels (Marcus and Schwartz, 1987).

Description of data. The Second National Health and Nutrition Examination Survey (NHANES II) was conducted from February 1976 until February 1980. Sampling was designed by the National Center for Health Statistics and the Bureau of the Census to be representative of the civilian noninstitutionalized U.S. population, aged 6 months to 74 years (National Center for Health Statistics, 1981). A total of 20,322 people were examined, and blood lead determinations were obtained for a subsample of 9932. Of these, 2981 women aged 20 or more years were selected for this analysis. The probability of selection for each individual was determined and the observations were weighted accordingly. Details of the complex survey design, the examination procedures, and the laboratory measurements have been published (National Center for Health Statistics, 1981; 1984). The medical evaluations included medical history, physical examination, anthropometric measurements, dietary information (24-hr recall and food frequency), laboratory tests, electrocardiograms, and radiographs. Special interview and examination protocols conducted by specifically trained interviewers and examiners were utilized to ensure the standardization of surveys conducted at each site. To calculate nutrient intakes, responses from the 24-hr recall questions were quantified for each individual using a current nutrient data bank.

Blood samples were analyzed by the Clinical Chemistry Division, Center for Disease Control, under an interagency agreement with the U.S. Food and Drug Administration. Details of the methodology and quality control for the various analyses have been published (National Center for Health Statistics, 1981, 1984). Blood lead concentrations were determined by atomic absorption spectrophotometry using a modified Delves cup micromethod (Barthel *et al.*, 1973). Both bench and blind quality controls were employed. Skinfold measurements were taken with calipers using standardized protocols, recorded to the nearest half millime-

ter. Weight was recorded by an automatic printing scale and measured to the nearest quarter of a pound. Height was measured in stocking feet with eyes fixed level in the Frankfort horizontal plane and determined from a photograph to the nearest millimeter. Body mass index (weight/height^2) was computed from these clinical measures. Menopause status, years since menopause, and prior pregnancy history were all determined from responses to the questionnaire given to all adult female examinees. Recreational exercise was coded at three levels in response to a question asking respondents to rate their own recreational activities as involving much, moderate, or little or no exercise. Of respondents 12 years and older (both male and female) 24% classified their exercise as much, 43% as moderate, and 33% claimed little or no exercise. The cigarettes per day variable was also determined from questionnaire response. A respondent was classified as an alcohol drinker if he or she responded affirmatively to drinking more than one glass of beer, wine, or liquor per week. Additional details on the questionnaire and other aspects of the survey are provided in the NCHS. In our preliminary analysis we first looked at the arithmetic mean blood lead levels for the 849 women aged 40–60, pre-and postmenopause. We then turned to multivariate analysis, controlling for possible confactors. Previous analyses have identified those variables that are associated with blood lead levels in the NHANES II survey (Barthel *et al.*, 1973; Mahaffey *et al.*, 1982; Annest *et al.*, 1983). These are shown at the top of Table 1. We also identified several variables related to the hypotheses that we were testing: menopause, (coded 1 for postmenopausal women, and 0 otherwise); years since menopause (entered as a continuous variable equal to the number of years or coded 0 for premenopausal women); and parity status (coded as 1 or 0). Race was also entered as a variable. Several variables related to calcium balance and bone mineral status were identified from the literature (Riggs and Melton, 1984; Cummings *et al.*, 1985; Ruegsegger *et al.*, 1984; Aloia *et al.*, 1985) and evaluated in the multivariate analysis. These were dietary calcium, recreational exercise, hypertensive medication, alcohol intake, and body mass index. These variables are all shown in Table 1.

Because the NHANES II survey is a stratified clustered random sample of the U.S. population, the design effects of the survey can lead to exaggerated significance levels for some variables. However, the distribution of pre- and postmenopausal women varies considerably across the country because of the large geographic variation in average age, independent of any sampling frame. Special statistical software has been developed to deal with the design effects in the context of NHANES II. We have used SURREGR, a procedure in SAS (SAS Institute, 1985; Shah, 1982; Survey Research Center Support Group, 1979). SURREGR adjusts for the presumed correlation between these outcomes and the sampling units as an effect of the stratification. In contrast, a purely random sample of the United States that was *post facto* divided into geographic areas would manifest a similar variation. If that variation were identical to the one in the NHANES II survey, then an ordinary least squares (OLS) regression would be more appropriate. Because we cannot definitively determine what proportion of the variation in menopause with the strata of the NHANES II survey would have occurred in a random survey, we have performed the analyses with both

TABLE 1
VARIABLES ENTERED IN UNIVARIATE AND MULTIVARIATE ANALYSES

Lead-related variables
Age (in years)
Age squared
Race[a]
Income[b]
Degree of urbanization[c]
Lead used in gasoline (10^9 g/day)
Number of cigarettes per day
Alcohol drinker (greater than one drink/week)
Variables related to osteoporosis
Dietary calcium (mg/day)
Hypertensive medication
Body mass index[d]
Subscapular skinfold (cm)
Dietary phosphorus (g/day)
Dietary protein (g/day)
Tricep skinfold (cm)
Recreational exercise[e]
Hypothesis variables
Menopause status
Years since menopause
Pregnancy history
Race

[a] 1 = black, otherwise 0.
[b] 1 = less than $5000/yr; 2 = $5000–15,000/yr; 3 = $15,000.
[c] 1 = cities over 3,000,000 to 8 = rural under 2500.
[d] weight/(height)^2.
[e] 1 = little or none; 2 = moderate; 3 = heavy.

SURREGR, and with REG, which is the standard regression procedure in SAS. We report both results.

RESULTS

The measured blood lead and calculated plasma lead levels pre- and postmenopause are shown for all women aged 40–60 years in Figs. 1a and 1b; for white women in Figs. 2a and 2b; and for black women in Figs. 3a and 3b, and the differences are described in Table 2. The data in Fig. 1a show significant increases in blood lead levels in postmenopausal, as compared to premenopausal, women. This increase was found in both blacks and whites, but the magnitude of change was smaller in black women (Figs. 2 and 3). To investigate the role of factors known or hypothesized to be associated with increased lead levels or bone status, multivariate analyses were then performed on these raw data. In addition, the analyses examined interactions between the postmenopausal increase in blood lead and these factors. To fully control for all these factors and maintain sufficient sample size, the age range was expanded to 20–74 years. All variables were eliminated by backward elimination and then each was tested individually in the final model to assure that they had not been dropped because of collinearity with

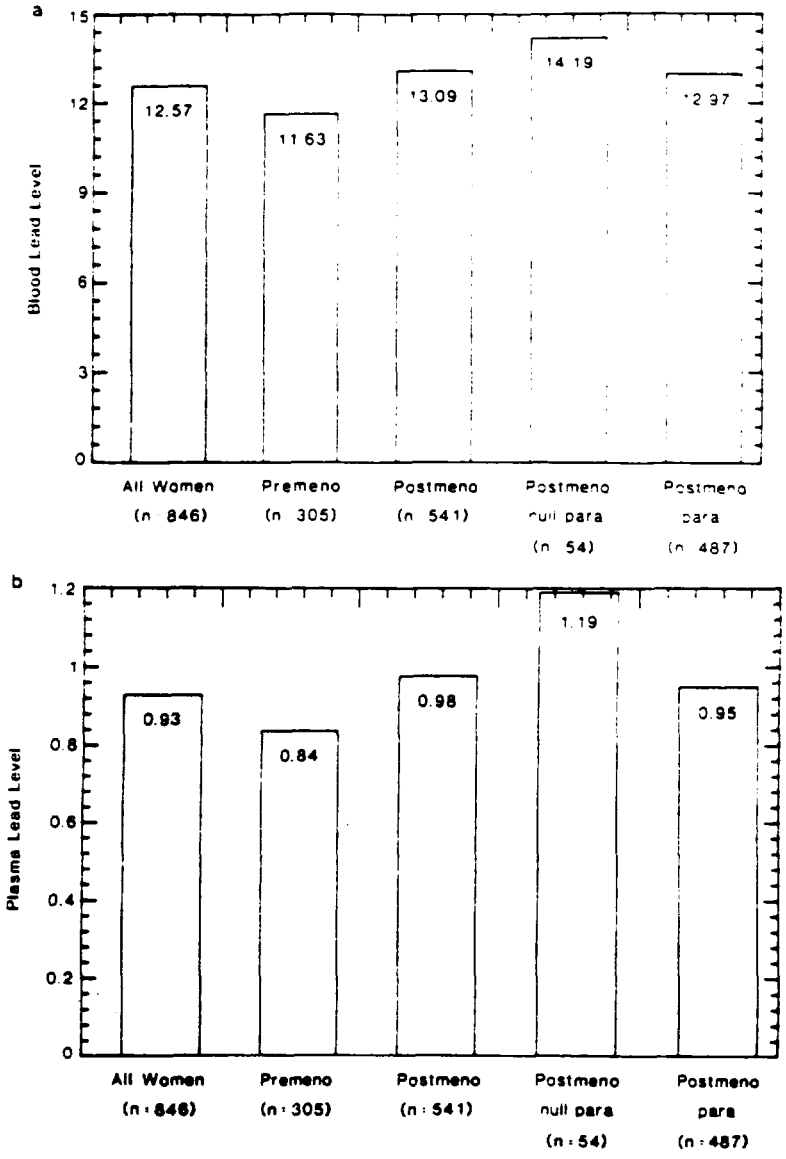

FIG. 1a. Blood lead levels in black and white women, aged 40 to 60 years. The bars denote, as follows (from left to right): all women (n = 846); all premenopausal women (n = 305); all postmenopausal women (n = 541); postmenopausal nulliparous women (n = 54); and postmenopausal women ever pregnant (n = 487).

FIG. 1b. Plasma lead levels in black and white women, aged 40 to 60 years. Bars are the same in Fig. 1a.

TABLE 2
EFFECT OF MENOPAUSE AND PRIOR PREGNANCY ON BLOOD AND PLASMA LEAD LEVELS IN WOMEN

	All women		White		Black	
	mcg/dl	% change	mcg/dl	% change	mcg/dl	% change
Change in blood lead						
pre/post meno	1.47	12.6%	1.67	14.7%	0.62	4.6%
pre/post meno never preg	2.56	22.0	2.67	23.4	1.31	9.8
pre/post meno ever preg	1.37	11.8	1.45	12.7	0.50	3.7
Change in plasma lead						
pre/post meno	0.136	16.2	0.150	18.3	0.030	3.0
pre/post meno never preg	0.347	41.3	0.386	47.2	0.104	10.3
pre/post meno	0.113	13.4	0.125	15.3	0.015	1.5

another insignificant covariate. The variables with statistically significant correlations to blood lead and predicted plasma lead levels are shown in Tables 3 and 4.

Womens' postmenopausal whole blood lead levels were significantly elevated after controlling for age and other relevant covariates. This increase declined with

TABLE 3
RESULTS OF WEIGHTED LEAST-SQUARES REGRESSIONS

	Coefficient	Standard error	*P* value
Dependent variable: blood lead			
Independent variable			
Gaslead	1.687	0.1025	0.0001
Age	0.1397	0.038	0.0002
Age squared	−9.7E-04	4.5E-05	0.0304
Race	0.838	0.270	0.0019
Income	−0.311	0.115	0.0067
Degree of urban.	−0.224	0.031	0.0001
Number of cigarettes	0.071	0.007	0.0001
Alcohol drinker	1.456	0.172	0.0001
Menopause	1.430	0.276	0.0001
Meno–Yr (MenoYr)	−0.0399	0.018	0.0229
Dependent variable: plasma lead			
Independent variable			
Gaslead	0.161	0.012	0.0001
Age	0.015	0.004	0.0007
Age squared	−9.7E-04	4.6E-04	0.0343
Race	0.065	0.032	0.0418
Income	−0.046	0.014	0.0007
Size	−0.017	0.0036	0.0001
Number of cigarettes	0.0057	8.3E-04	0.0001
Alcohol drinker	0.134	0.020	0.0001
Menopause	0.294	0.053	0.0001
MenoYr	−0.006	0.002	0.0058
Meno–Preg	−0.166	0.045	0.0002

Using the more conservative SURREGR model gives similar results for both the impact of menopause and years since menopause on blood lead levels. However, with SURREGR, for the predicted plasma lead, the variables race, number of years since menopause, and the pregnancy interaction term were not significant. The coefficient of the menopause term indicates that in the first year of menopause blood lead levels are elevated by an average of 1.4 mcg/dl above what they otherwise would have been in premenopausal women controlling for age, race, income, alcohol consumption, and other variables as shown in Tables 2 and 3.

DISCUSSION

The finding of elevated blood lead concentrations in a national sample of postmenopausal women, as compared to menstruating women, controlling for age, is consistent with the hypothesis that physiological conditions which cause mobilization of bone calcium will also cause mobilization of bone lead (Pounds, 1984; Rosen and Wexler, 1977; Rosen, 1983b; Pounds and Rosen, 1986). The overall increase in lead levels in postmenopausal women could have been discerned in the age trend analyses reported earlier (Mahaffey *et al.*, 1982). In that report, the median blood levels for women tend to peak at about the median age for menopause. It should be noted that in this study we could only identify women by menstrual status. No information was available as to how many of the postmenopausal group actually may have had clinical osteoporosis. Thus the results for the group as a whole are an average between women with and without osteoporosis; blood lead levels in postmenopausal *osteoporotic* women are likely to be even more elevated, as compared to premenopausal, nonosteoporotic women.

It is possible that factors other than menstrual status are associated with this observation. However, since statistical analyses controlled for age, the finding is unlikely to be a cohort effect, related to earlier and probably higher environmental exposures to lead. Moreover, the postmenopausal increases did not significantly vary with the risk factors which are identified with increased lead absorption, such as race, income, and residence (population of place of residence). This also suggests that these increases are not related to variables which relate to *external* exposures to or absorption of lead concurrent with menopause.

The postmenopausal increases in blood lead declined over time, similar to the temporal pattern which has been described for bone resorption (Cummings *et al.*, 1985). This parallel suggests that similar physiological processes are involved in both osteoporosis and increased blood lead levels. Increases in blood lead levels in postmenopausal women were not related to those risk factors which have been associated with osteoporosis, such as calcium intake in diet, exercise, alcohol, or caffeine consumption (see tables). The association of these factors with postmenopausal osteoporosis is weak (Riggs and Melton, 1984; Cummings *et al.*, 1985), which may explain their lack of association with increased lead levels.

The postmenopausal increase in lead levels was less in women with prior pregnancies (Figs. 1, 2, and 3). This observation is consistent with the hypothesis that pregnancy also causes bone demineralization, resulting in less lead available for mobilization in the postmenopausal period. Moreover, nulliparity has been hy-

pothesized to be a risk factor for osteoporosis, so that women with prior pregnancies may have experienced less bone demineralization (Riggs and Melton, 1984). This hypothesis assumes that similar mechanisms are involved in both conditions and that the same sites in bone are affected by pregnancy and menopause, which is not unreasonable, since a limited area of bone is involved in resorption (Arlot *et al.*, 1984).

Postmenopausal increases in blood lead were observed in both black and white women (Hispanics were not sampled heavily enough for separate analyses in NHANES II; they are being studied for lead status in the Hispanic NHANES). However, the increase was relatively much greater for white women (Table 4; Figs. 2 and 3). This is of interest, since blood lead levels are, on average, higher in blacks than in whites (Mahaffey *et al.*, 1982). However, postmenopausal osteoporosis has a significantly lower incidence in black women (Cummings *et al.*, 1985). Thus the pattern of postmenopausal changes in blood lead levels follows that of osteoporosis rather than lead exposure. The effect of prior pregnancy was seen in both black and white women, although much greater relative effects (to decrease the postmenopausal change) were seen in blacks. In white women, prior pregnancy reduced the postmenopausal increase in blood lead by approximately twofold; in blacks, the effect of prior pregnancy was to reduce postmenopausal changes in blood lead by almost threefold (Table 4). (In multivariate analysis, these effects were of a similar magnitude, although marginally insignificant.) This may be due to the greater average number of prior pregnancies in black women in this sample (mean = 4.12) versus whites (mean = 3.43). This could not be examined because we could not assess the impact of parity further (e.g., the effect of number of pregnancies) because of insufficient sample size. Blacks and whites may not differ in response to pregnancy and menopause, in terms of lead mobilization. Alternatively, the effect of pregnancy on mineral mobilization may not vary by race, while the extent of postmenopausal demineralization may vary in some way, which might be consistent with the observed lower incidence of clinical osteoporosis in black women (Riggs and Melton, 1984; Cummings *et al.*, 1985).

Independent of the significance of the pregnancy variable in this analysis, the significance of the menopause variable suggests that lead may be mobilized during pregnancy. Bone lead mobilization has been found to occur in mice, during pregnancy and lactation (Keller and Doherty, 1980). In two women, significant loss of bone lead was measured during pregnancy; in one case, the subject (who had had clinical lead poisoning in childhood) expressed overt signs of lead toxicity during pregnancy (Manton, 1985; Thompson *et al.*, 1985). The toxicological significance of such mobilization during pregnancy may be substantial in light of recent information on the adverse effects of lead on reproduction and development in young children (Dietrich *et al.*, 1987; Needleman *et al.*, 1984; Bellinger *et al.*, 1984, 1986; 1987; McMichael *et al.*, 1986).

It is also possible that lead may have some etiologic role in osteoporosis. A major unifying mechanism of lead toxicity in liver, brain, and bone cells is perturbation of intracellular molecular calcium regulation (Pounds, 1984; Pounds and

Rosen, 1986; Silbergeld, 1985). Experimental work in primary cultures of murine bone cells confirms the hypothesis that lead–calcium interactions also occur in this tissue (Rosen and Wexler, 1977; Rosen, 1983a; Pounds and Rosen, 1986). The balance between osteoblast and osteoclast activity may be critical in postmenopausal osteoporosis (Gruber *et al.*, 1986). Lead-induced perturbations of calcium homeostasis may affect the regulation of cell calcium by calciotropic hormones through changes in intracellular ionized calcium concentrations (Edelstein *et al.*, 1984; Rosen, 1983a; Pounds and Rosen, 1986). Lead inhibits osteocalcin binding to hydroxyapatite *in vitro* and displaces calcium from high affinity binding to osteocalcin (Markowitz *et al.*, 1986). A recent study reported effects of lead on calcium efflux from rat pituitary cells *in vitro*, and an inhibition of thyroid-stimulating hormone release elicited by thyrotropin-releasing hormone (Huseman *et al.*, 1987). In experimental models of lead toxicity, lead exposure *in vivo* primarily inhibits bone formation (Anderson and Danylchuk, 1977; Hass *et al.*, 1967). Lead also impairs calcium uptake from the gut (Gruden, 1975) and inhibits the hydroxylation of 25-hydroxyvitamin D to the active hormone, 1,25-dihydroxyvitamin D (Mahaffey *et al.*, 1982). In children with blood lead levels between 12 and 120 mcg/dl, lead in blood was negatively correlated with concentrations of 1,25-dihydroxyvitamin D in serum (Rosen *et al.*, 1980; Mahaffey *et al.*, 1982). These clinical observations have been confirmed directly in experimental studies (Edelstein, 1984). These effects of lead may partly explain the relatively lower postmenopausal increases in blood lead observed in black women in this study: as compared to whites, blacks have been reported to have increased levels of 1,25-dihydroxyvitamin D and enhanced renal reabsorption of calcium (Bell *et al.*, 1985).

The NHANES II data used in this study do not allow for direct examination of potential interactions between body lead burdens and the development of osteoporosis. Nevertheless, based upon the data available concerning the pathophysiologic effects and mechanisms of lead, it is conceivable that lead may play a role in osteoporosis (postmenopausal and/or senile type) (Riggs and Melton, 1984)).

Because of the prevalance of osteoporosis and the predicted growth in the numbers of older women at risk for osteoporosis (Riggs and Melton, 1984; Cummings *et al.*, 1985), investigation of possible risk factors is important. Unfortunately, undue lead absorption remains quite prevalent in the U.S. population (Mahaffey *et al.*, 1982), so that the study of lead and osteoporosis is feasible. It is in fact planned for the next NHANES survey.

In summary, these findings are of interest for two reasons: First, they demonstrate that bone lead can be mobilized, such that significant amounts of lead may be transferred from bone to more bioavailable compartments. Second, this mobilization may have toxic implications for at least two groups: the perinate, who may be exposed *in utero* and during nursing to lead in maternal circulation which is derived from both concurrent and past maternal exposure, and the older woman at risk for osteoporosis. The toxicological consequences in older women of relatively rapid increases in internal lead dose are unknown.

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